

10 We claim:

1. A cardio myopeptidin, which is a polypeptide isolated from hearts of healthy non-human mammals, comprising 75%~90% of peptide, 6% ~ 15% of free amino acid, less than 2% of ribonucleic acid, less than 7.5% of deoxyribonucleic acid, and molecular weight is less than 10000 Da.

15 2. The cardio myopeptidin of claim 1 wherein said non-human mammals comprises pigs, cattle, sheep, rabbits or horses; preferably the infant mammals, and more preferably infant pig.

3. The cardio myopeptidin of claim 1 wherein average molecular weight is in the range from 1000 to 10000 Da, preferably from 2000 to 8000Da, and more preferably from 2000 to 5000Da.

20 4. The cardio myopeptidin of any one of claim 1 to 3 wherein the biological activity of cardio myopeptidin is stable at pH from 3 to 8, the cardio myopeptidin is sensitive to protease K, the biological activity will not change at the temperature of 85°C for 10 minutes, and is stable under frozen or lyophilized condition.

5. The cardio myopeptidin of any one of claim 1 to 3 wherein isoelectrofocusing electrophoresis of said cardio myopeptidin displays 2~6 stained bands, preferably two bands, among which, the band whose pI is 10.92 is the one with deeper color.

25 6. The cardio myopeptidin of any one of claim 1 to 3 wherein said cardio myopeptidin has a stable maximum absorption peak at 190~210 nm wavelength in UV spectrum, preferably the maximum ultraviolet absorption peak at 200±2 nm wavelength.

7. The cardio myopeptidin of any one of claim 1 to 3 wherein the activity of said cardio myopeptidin is 2.2.

30 8. The cardio myopeptidin of any one of claim 1 to 3 wherein said cardio myopeptidin further comprises excipient, and the content by weight is:

cardio myopeptidin: 15~20

Excipient: 100~375 ,

preferably the content is 18~20: 200~375.

35 and the excipient is mannitol, trehalose, lactose or sucrose or other adjuvants for lyophilization; preferably mannitol.

9. The cardio myopeptidin of any one of claim 1 to 3 wherein said cardio myopeptidin further comprises activated carbon with the content from 0.05% to 0.1%.

40 10. The cardio myopeptidin of any one of claim 1 to 3 wherein said cardio myopeptidin show five peaks on FPLC analysis spectrum, and the sum of relative area is 90%~95%.

11. A method for the preparation of cardio myopeptidin in claim 1 comprising the step of:

(a) cleaning and cutting the hearts of healthy non-human mammals;

(b) homogenizing by adding sterile distilled water to the myocardium of healthy non-human mammal which is cleaned and cut;

45 (c) freezing and thawing cycles the homogenate by alternately for 3 or 4 times;

(d) filtering by the plate-and-frame filter to get a coarse filtrate and removing the residue after the homogenate is heated to 65~95°C;

(e) ultra-filtering the coarse filtrate with a hollow-fiber column to get a fine filtrate;

50 (d) ultra-filtering the fine filtrate by ultrafiltration membrane to intercept the cardio myopeptidin solution with the molecular weight less than 10000 Da;

(e) concentrating the solution by reverse osmosis to get a concentrated cardio myopeptidin solution.

(f) testing the quality, filtrating aseptically, filling and lyophilizing.

12. The method of claim 11 wherein amount of sterile distilled water added is from 0.5 to 4 times of that of the myocardium of mammals, and the rotation speed of homogenization is in the range from 1000 to 5000 rpm/min.

13. The method of claim 11 wherein said freezing is performed at a temperature of less than -5°C for 24~72 hours, preferably at -20°C~-30°C for 36~48 hours; heating is in the way of water bath heating or direct heating at a temperature of 70~90°C for not more than 2 hours, and preferably water bath heating at a temperature of 75°C~80°C for 1 hour.

14. The method of claim 11 wherein said the plate-and- frame filter comprise medium-speed filter paper having pores less than 10μ, preferably the pores less than or equal to 5μ; fine filtrate with molecular weight less than 12k Da is obtained through a hollow fiber column, and final filtrate with molecular weight less than 10k Da is obtained by intercepting part of solution through ultrafiltration membrane.

15. The method of claim 11 wherein the process of lyophilization comprises the step of: the shelf in the drying chamber is cooled down to the temperature of -15°C~-20°C in 5~40 minutes, preferably to -18°C~-20°C in 20~30 minutes, followed the cardio myopeptidin is frozen to the temperature of -25~-35°C within 20~40 minutes and maintaining at this temperature for 1~3 hours, preferably to -30~-35°C within 25~35 minutes; then the condenser is chilled to the temperature of -40~-50°C; at that time the pressure is reduced till the vacuum degree reaches 90~100 Kpa, the drying chamber is connected with condenser, and the refrigeration is stopped; after that, the temperature of drying chamber is raised to 5~15°C at the rate of 2~5°C/min and maintained at this temperature for 3~6 hours when vacuum degree of the drying chamber gets to 10~15 Pa, preferably the temperature is ascended to 8~12°C at the rate of 3~4°C/min with 4~5h heat preservation; the temperature is elevated continuously to 15~25°C at the rate of 8~16°C/min and kept for 3~8 hours, preferably the temperature is raised to 18~22°C at the rate of 10~12°C/min for 4~6 hours; then the temperature is further increased continuously to 30~35°C at the rate of 7~15°C/min and maintained for 1~4 hours, preferably 33~35°C at the rate of 9~12°C/min for 1.5~2 hours; furthermore the temperature is raised continuously to 50~60°C at the rate of 4~8°C/min for lasting 1~3 hours, preferably to 54~58°C at the rate of 5~7°C/min for 1.5~2 hours; then come to the cooling stage, in which the temperature is cooled down to 40~50°C within 10~30 minutes and stood such temperature for 8~15 hours, preferably cooled down to 45~48°C in 15~20 minutes and 9~12h preservation at such temperature to attain lyophilized production of cardio myopeptidin with qualified appearance.

16. The use of cardio myopeptidin of claim 1 for the manufacture of a medicament for the treatment of cardiovascular disease.

17. the use of cardio myopeptidin for the manufacture of a medicament for the treatment of myocardial ischemia-reperfusion injury.